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Predicting *Lemna minor* growth rate response to temperature fluctuations

by

Elizabeth Novich

AN UNDERGRADUATE THESIS

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Abstract

The effects of global climate change on populations may be further understood when environmental variability is included. There are strong theoretical reasons that support the idea that environmental fluctuations matter when studying ecological systems. In order to further explore this, we parameterized a model of the growth rate of *Lemna minor* at constant temperatures and made predictions on how *L. minor* would respond to fluctuating temperatures based on this model. There were four trials performed that compared the difference between growth rates at constant and fluctuating temperatures in order to test if our predictions were correct. For our results, only the high temperature treatment with sine wave fluctuations proved to match our predictions significantly, which could be due to the simplicity of our model from which we made predictions. Our results suggest that in order to fully describe how populations respond to environmental variability, we may need to find a more complex model.

Introduction

Fluctuations in environmental conditions can be a key factor to include when studying population growth rates in realistic ecological contexts. Historically, a large number of studies have focused on a constant environment when describing population dynamics (Ruel & Ayres 1999). This pattern can be observed in current studies regarding global climate change. When studying the effects of global climate change on population dynamics, many scientists focused on organism responses to mean values (Estay 2010). Research confirms that the global temperature has increased by about 0.6°C over the past century (Walther 2002). One recent study of the effects of climate change on a population of great tits (*Parus major*) showed that their mean egg-laying date has advanced by about 14 days over the span of 47 years

(Charmantier et al. 2008). Global Climate change could affect species abundance, distribution, life cycle timing, growth, physiology and productivity (Hughes 2000). Certain species or geographical areas will be more affected than others (Kingsolver 2009) due to the various responses and adaptive abilities of certain species and the different diurnal ranges of certain locations (Walther et al. 2002). However, these studies have not included the fact that environments are variable. Variability in temperature and precipitation is predicted to increase based on predictions of climate change models (Hughes 2000). Extreme weather events could also increase, such as heat waves, cold waves, heavy precipitation, drought, and tropical storms (Easterling et al. 2000). Therefore, more studies need to focus on environmental variability to see the effects of this increased variability on populations. The purpose of this study was to examine the consequences of environmental variation on *Lemna minor* (duckweed) and provide insight on how population dynamics might be affected by climate change.

There are strong theoretical reasons that support the idea that environmental fluctuations should matter for ecological systems. One is the mathematical principle known as Jensen's inequality, which can be used to describe and predict the consequences of environmental variation (Ruel & Ayres 1999). Jensen's inequality states that nonlinear responses will be elevated or depressed from the response to constant conditions when there are fluctuations in that condition. The concavity of these responses will determine whether it will be elevated or depressed. If the curve is concave up, then fluctuations will elevate the growth rate and if it is concave down, then fluctuations will depress the growth rate.

Nonlinear responses are common in biological systems. Most biological processes in organisms display the same thermal response as biochemical reaction rates, which are generally nonlinear and asymmetrical below the optimum temperature. In terms of concavity, growth rate

will be concave up at low temperature ranges and concave down at high temperature ranges (similar to Figure 1). This recurrent shape can be used to recognize and understand patterns of variation in the fitness of many different organisms (Kingsolver 2009). Temperature is only one example of a nonlinear response in biological systems. There are many other examples of nonlinearity in biological systems, from responses to light variability in photosynthetic organisms to variable qualities in hosts for herbivorous organisms (Ruel & Ayres 1999).

Some current research has incorporated variability. One recent study measured the maximum per capita growth rate of flour beetles (*Tribolium confusum*) at constant and fluctuating temperatures. It was found that there was a significant difference between the maximum per capita growth rates in constant versus fluctuating temperatures (Estay et al. 2010). This result provides evidence that fluctuations should be incorporated into studies of population dynamics and environmental variation. Another study tested the effects of temperature variability on body mass, growth rate, survival, metabolic rate and molecular traits in a terrestrial woodlouse (*Porcellio laevis*). They concluded that many studies that use only mean temperatures to predict the impact of global climate change ignore many complex molecular and physiological processes used by organisms to deal with environmental variability (Folguera et al. 2011).

However, these studies have overlooked possible asymmetrical responses to these conditions whereas in our study we will look specifically at this asymmetry. This study addressed the question of how nonlinear asymmetrical responses to environmental conditions affect population dynamics in fluctuating environments. Our prediction was that the growth rate of duckweed populations will be elevated when there are fluctuations around a low temperature and depressed when there are fluctuations around a high temperature. This prediction was based off of Jensen's inequality and the constant temperature growth curve. For this study, we tested

this prediction by first measuring the growth rate of *Lemna minor* populations at a range of constant temperatures. The results were then be fitted to a model, and the concavity of this model allowed us to predict whether temperature fluctuations at certain temperatures will be elevated or depressed from the per capita growth rate at stable temperatures. After measuring the growth rate at constant temperatures, we then tested the growth rate in fluctuating temperatures in order to evaluate the importance of Jensen's inequality in a real population.

Materials and Methods

Experimental System

In order to test the effects that temperature fluctuations have on population dynamics, we used the species *Lemna minor*. This floating aquatic plant, commonly known as duckweed, was useful for this experiment because it has a high growth rate and is easy to maintain in the laboratory microcosms. *L. minor* reproduces asexually with a generation time of 3-5 days (Wynn & Brassil unpublished). The strain used for our experiments was collected in Memphis, Tennessee.

Experimental Setup

In order to make precise predictions about how populations will respond to temperature fluctuations, we used a mathematical model that described growth rate as a function of temperature. We constructed a curve specific to our strain and growth conditions. We measured the per capita growth rate of *L. minor* at set constant temperatures. Data was previously collected in the lab on the growth rate response to different constant temperatures (Phillips and Hadan unpublished), and so we focused on collecting more data points at low temperatures and other areas in order to complete the constant temperature growth curve. To accomplish this, 40

cups were placed in the 6 different temperature chambers set at the following temperatures: 7.5, 10.0, 12.5, 15.0, 20.0 and 27.5°C.

This parameterized model allowed us to predict the response of duckweed populations at fluctuating temperatures based on the concavity of the curve (Figure 1). Based on our qualitative predictions, we designed experiments with temperature fluctuations at low and high temperatures. The mean temperatures for the trials were 28 and 13°C, which were chosen due to the different concavities at these points on the growth curve (28 in the concave down region and 13 in the concave up region). Also, 28°C was chosen because it is approximately the optimal temperature for the growth rate of duckweed. For these trials, there were 20 cups with duckweed in every chamber. In the first trial, the growth of duckweed at a constant temperature of 28°C was compared to the growth of duckweed with sine wave temperature fluctuations between 22°C and 34°C with a mean of 28°C (Figure 2). For the second trial, we compared the growth of duckweed between a constant temperature of 13°C and sine wave fluctuations between 7°C and 19°C with a mean of 13°C (Figure 3). In both of the previous trials, there were 3 chambers with constant temperatures and 3 chambers with sine wave fluctuations. In order to see if the results were robust to more realistic temperature fluctuations, a third trial was run that compared the growth of duckweed at a constant temperature of 28°C to stochastic temperature fluctuations with a mean of 28°C that were pink-shifted. This type of noise was chosen because it is more similar to natural temperature fluctuations than white noise (Stoyanow et al. 2011). The fourth trial was similar to the third trial except the constant temperature and the mean temperature of the stochastic fluctuations was set to 13°C. The variance for the noise for both trials was between 17 and 19 and temperatures never went below 0°C. There were 4 chambers with stochastic fluctuations and 2 chambers with constant temperatures for the third and fourth trial. For the

stochastic fluctuations, the chambers were set to change temperature every half hour whereas for the sine fluctuations, the temperature was set to change every 4 hours. The Intellus software was set to ramping mode to allow continuous temperature changes.

Each of the trials was conducted across six Percival chambers with Intellus Software. These temperature chambers were set at 65% humidity. The average PAR reading for the chambers was $91.78 \mu\text{mol}\cdot\text{s}^{-1}\text{m}^{-2}$, which was measured using a LI-COR light sensor (model LI-250A Light meter). The light schedule was set for 16-hours light, 8-hours dark. Duckweed populations were maintained without algae or blue-green algae contamination by following modified axenic techniques (Bowker et al. 1980). The duckweed was grown in 100mL of Swedish Standard Growth Medium (OECD 2006). Complete nutrient replacement was performed every 3.5 days to ensure that there wouldn't be a lack of nutrients that would negatively affect the growth rate. About 11-13 fronds of duckweed were added to these cups from axenic cultures. The fronds were allowed to acclimate to the set temperature in the chambers for a week, after which we removed 11-13 fronds from each cup and transferred the fronds to new cups that were placed in the same chamber respectively. For the temperature fluctuation experiments, the temperatures were constant for the first week and set to fluctuate during the second week. For the second week, two HOBO Data loggers were placed in two cups each with water in every temperature chamber in order to record the temperature of each chamber every 5 minutes. The initial and final population sizes at the beginning and end of the second week and were subsequently counted by hand from digital photos using a frond marker program written in Mathematica. A frond was counted if it was visible in the photograph.

Data Analysis

In order to parameterize the constant temperature growth curve, we calculated the growth rate (r) of the duckweed populations. This was calculated assuming exponential growth of the duckweed, due to the excess in nutrients to prevent negative density-dependence. The initial population size was also chosen to be small enough so that the duckweed would not be limited by space. By solving the continuous time equation for exponential population growth, we get the following expression for (r):

$$r = \frac{\ln(N_t) - \ln(N_0)}{t} \quad (1)$$

where t is time, N_t is the final population size (at time t) and N_0 is the initial population size (at time 0). The time interval was around 7 days (time interval measured to the nearest hour).

After calculating the growth rate of each duckweed population at the different constant temperatures, we used these results to parameterize the following constant temperature growth curve suggested by Van der Heide (2006).

$$\begin{aligned} &g(T) \\ &= \frac{r_{max} (T - T_{min})}{(T - T_{min}) + (T_{opt} - T_{min}) e^{-\frac{(T - T_{opt})^2}{k}}} \end{aligned} \quad (2)$$

The temperature values used in this equation were the measured temperatures from the HOBO loggers rather than the set temperatures. To make quantitative predictions, we calculated the average growth rate over the duration of the experiment at every 5 minute interval of the measured temperatures for a list of growth rates. We then calculated the total growth and

divided it by the number of points where growth was calculated, and then divided it by the time period to the nearest hour (around 7 days) (Appendix II).

In order to test whether the growth rates were significantly different between constant and fluctuating temperatures, a nested Analysis of Covariance (ANOVA) was used in R. This statistical test was used in order to avoid pseudo replication because it accounts for the fact that the cups are grouped in chambers. The measured temperatures were used as the covariate.

Results

The resulting constant temperature growth curve had these values for equation 2: $R_{max}=0.455$, $T_{opt}=29.621$ and $T_{max}=39.000$ (Figure 1).

The per capita growth rates of duckweed grown in the sine wave fluctuating temperatures were significantly lower than the per capita growth rates in constant temperatures at 28°C (Figure 4) ($p=0.004$, $F=35.361$, $df=4$, sine mean=0.438, constant mean=0.407). The per capita growth rates of duckweed populations in the sine wave fluctuating temperatures for the cooler temperature (13°C) were the same as the growth rates at constant temperature (Figure 5) ($p=0.532$, $F=0.466$, $df=4$, sine mean=0.092, constant mean=0.099). For the stochastic fluctuations with a mean of 28°C, the per capita growth rates were the same as the growth rates at constant temperatures (Figure 6) ($p=0.723$, $F=0.145$, $df=4$, random mean=0.442, constant mean=0.437). The per capita growth rates were the same as the stochastic fluctuations with a mean of 13°C than the growth rates at constant temperatures (Figure 7) ($p=0.583$, $F=.356$, $df=4$, random mean=.097, constant mean=.105).

Discussion

According to our analysis, the growth rates in sine wave fluctuations at high temperatures were significantly lower than at a constant temperature whereas the growth rates at cooler fluctuations were not significantly lower than at constant temperature. Our results also show that the growth rates were significantly lower for the sine wave fluctuations and were not significantly lower in the stochastic fluctuations at high temperatures.

Since our predictions were not correct for the low temperature sine wave fluctuations trial and the two stochastic fluctuation trials, this could mean that the equation we used for our constant temperature growth curve does not fully describe the growth rate of the duckweed and is too simple to explain how duckweed responds to temperature fluctuations. The constant temperature growth curve doesn't line up exactly with the data points, as it is above most of the data points at cold temperatures (Figure 1). Therefore, we were unable to make accurate predictions based on our model. An interpolation was performed in order to further analyze this growth curve, from which we performed more quantitative predictions (Appendix II). In future research more analysis will be performed in order to have a better understanding of the results.

One possible reason that the responses to stochastic fluctuations were not significant compared to the sine wave fluctuations could be that the fluctuations were too rapid for the duckweed to respond. It is possible that the temperature of the media had delayed or buffered fluctuations due to the differences in the amount of time it takes to change air temperature verses water temperature. This might explain why the duckweed was able to respond to the sine

fluctuations, because they were relatively slow compared to the stochastic fluctuations (Appendix III for actual temperatures).

It is interesting to note that the duckweed response to the sine wave temperature fluctuations was significantly lower from the response to constant temperature for the high temperature but it was not significant for the low temperature. We had predicted that the growth rate would be elevated when fluctuations were added. It is possible that the duckweed populations had lower growth rates in sine wave fluctuating temperatures due to the cost of physiologically adjusting to the ever-changing environment which leveled out the predicted elevation. The study conducted by Estay et al. (2010) that measured the effects of temperature fluctuations on the growth rate of flour beetles hypothesized that organisms that live in fluctuating environments incur a metabolic cost due to the energy spent towards adaptation (Estay 2010). This implies that the energy that would have gone towards reproduction is now being used to acclimate to the fluctuating environment. Their study showed that the maximum growth rate in step-change fluctuations at warm temperatures were significantly lower than the maximum growth rate at constant temperatures, which was similar to our result for the sine wave fluctuations at 28°C. However, they did not test the response to temperature fluctuations at cold temperatures.

Another possible explanation why growth rates at cooler sine wave fluctuations were not significant could be due to the fact that metabolic processes run slower at cooler temperatures than at warmer temperatures. Therefore, maybe we should have increased the time period the duckweed was in the chambers in order to get the predicted response. Also, if we had chosen a higher amplitude, then the fluctuations would be further away from a constant temperature, which could show a more significant difference between fluctuating and constant temperatures.

If we had chosen a lower amplitude, the fluctuations would be more similar to constant temperatures. Therefore, it is possible that if this experiment had a different amplitude or period our results might have been different.

In order to further explore predicting growth rate response to temperature fluctuations, we will need to find a mathematical model that describes the duckweed growth accurately in constant temperatures. For another experiment, we could try to change the amplitude or period to see how that may affect the results. It may be difficult to find a model that accurately describes the growth of duckweed, however if we were to find such a model it could have many potential applications. For example, many studies that want to include environmental variability would be able to make precise predictions about a population's response to this environment. This could be useful in studies that want to predict how populations will respond to the effects of global climate change.

Acknowledgements

I would like to express my gratitude my thesis advisor Chad Brassil for all of his help and guidance during this process. I would also like to thank Brigitte Tenhumberg for revising my undergraduate thesis. Also, I am grateful to Joseph Phillips for his help on the experiments and analysis. I would also like to express thanks to Cale Hadan for maintaining the laboratory and axenic cultures. I could not have completed this project without all of your help. Thank you.

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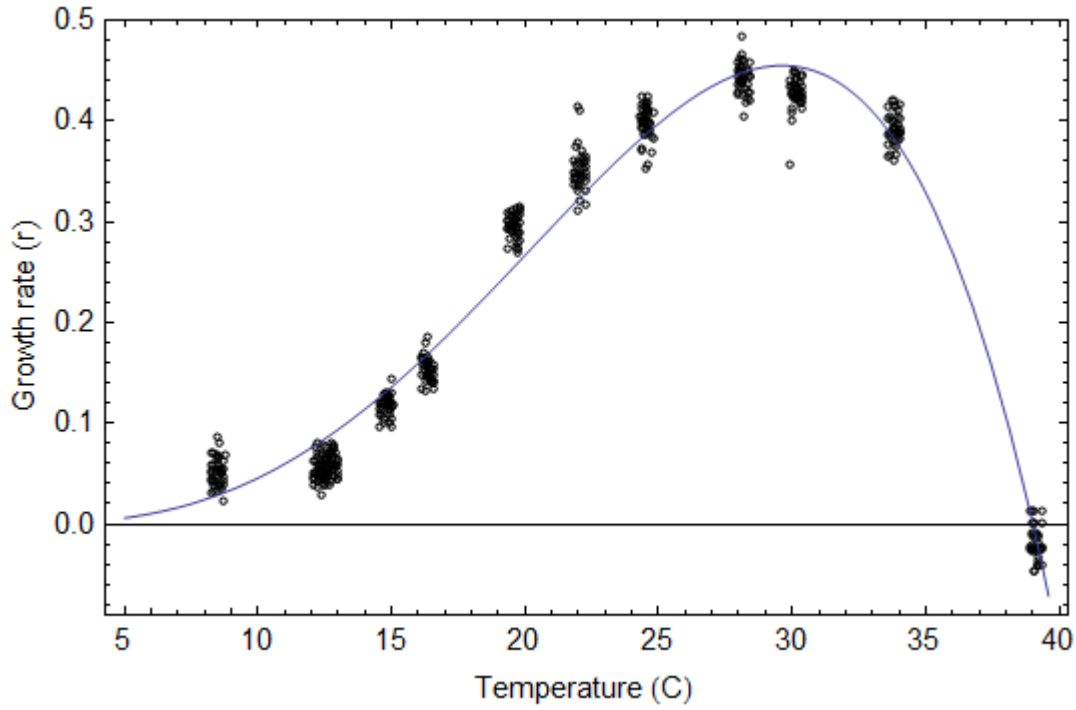


Figure 1. This constant temperature growth curve was parameterized by using equation 2 from Van der heide (2006). Each dot represents the growth rate of one population of duckweed, or one cup. Some of the data points for this figure were collected previously (Phillips and Hadan unpublished).

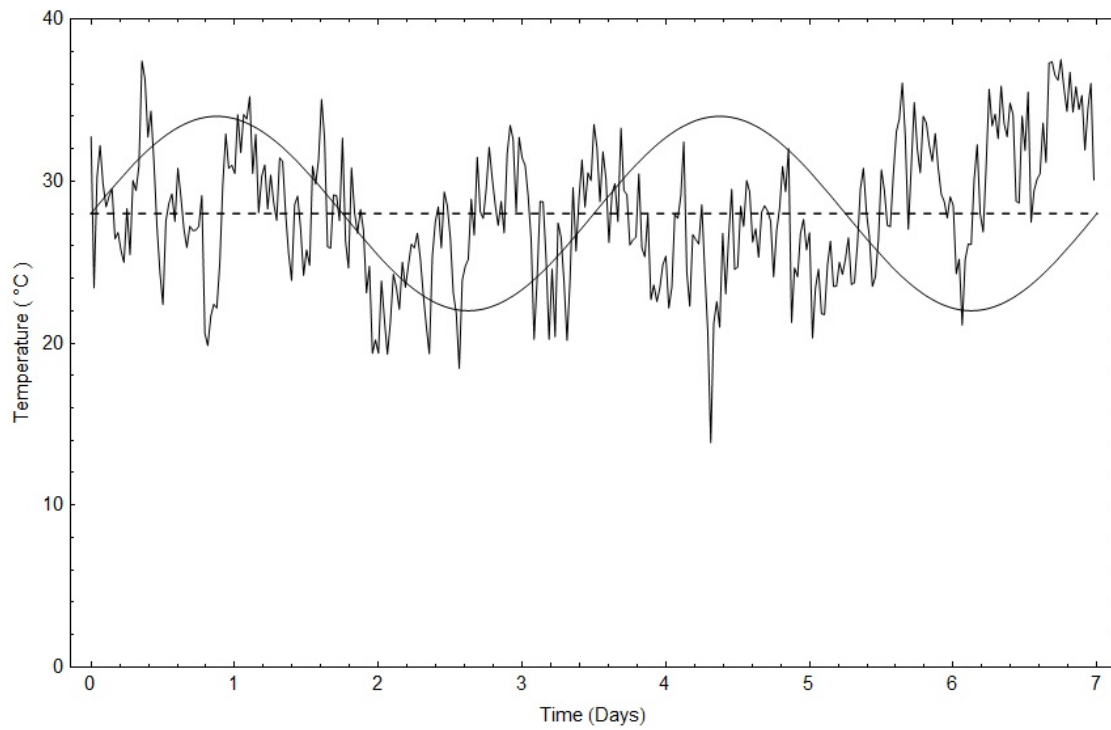


Figure 2. The figure shown above is an example of the sine wave and stochastic temperature fluctuations around 28°C (this is based on set temperatures).

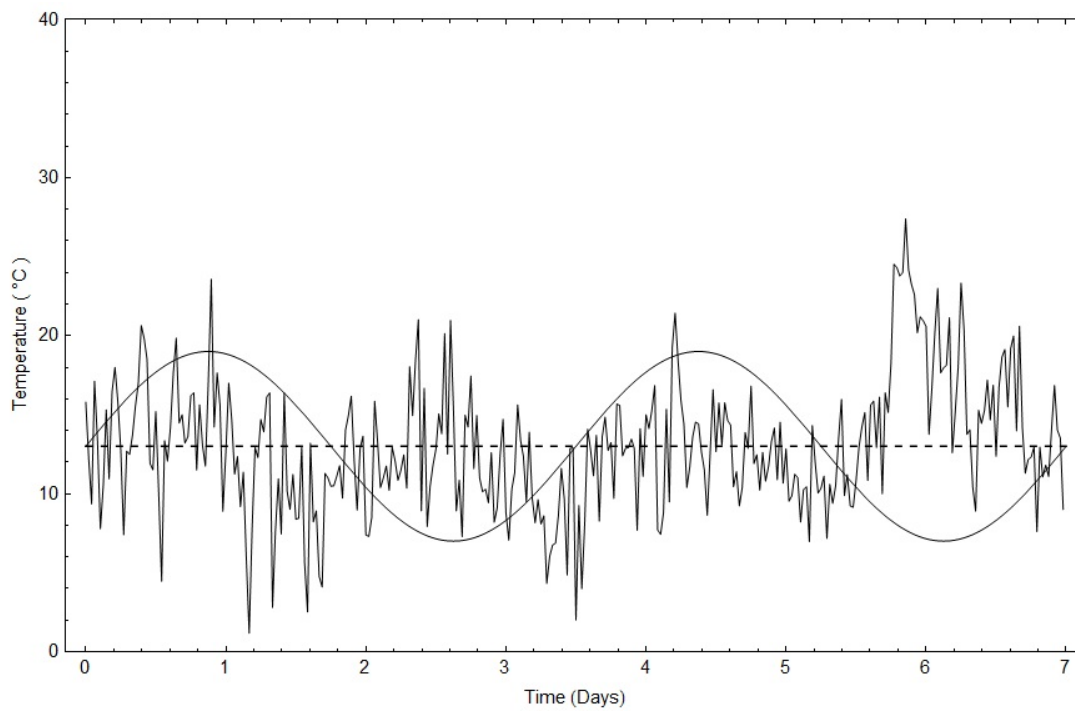


Figure 3. The figure shown above is an example of the sine wave and stochastic temperature fluctuations around 13°C (based on set temperatures).

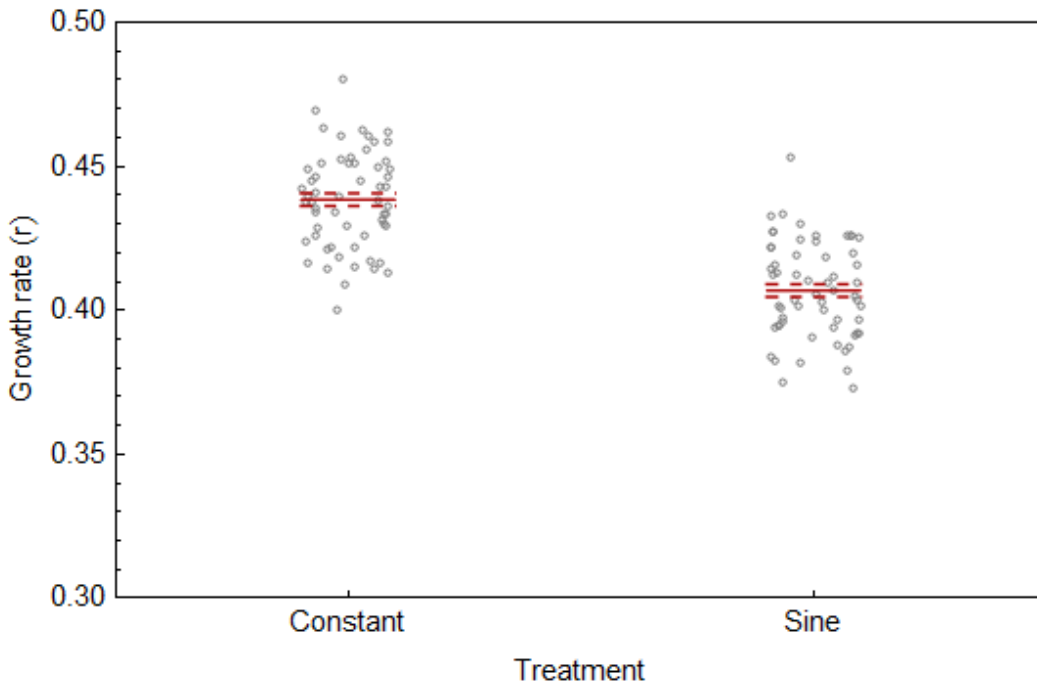


Figure 4. This figure compares the growth rates at constant 28°C and at sine wave fluctuations around 28°C. Each dot represents one duckweed populations, or one cup. The solid middle line is the mean of the growth rates, while the dotted lines represent the standard error.

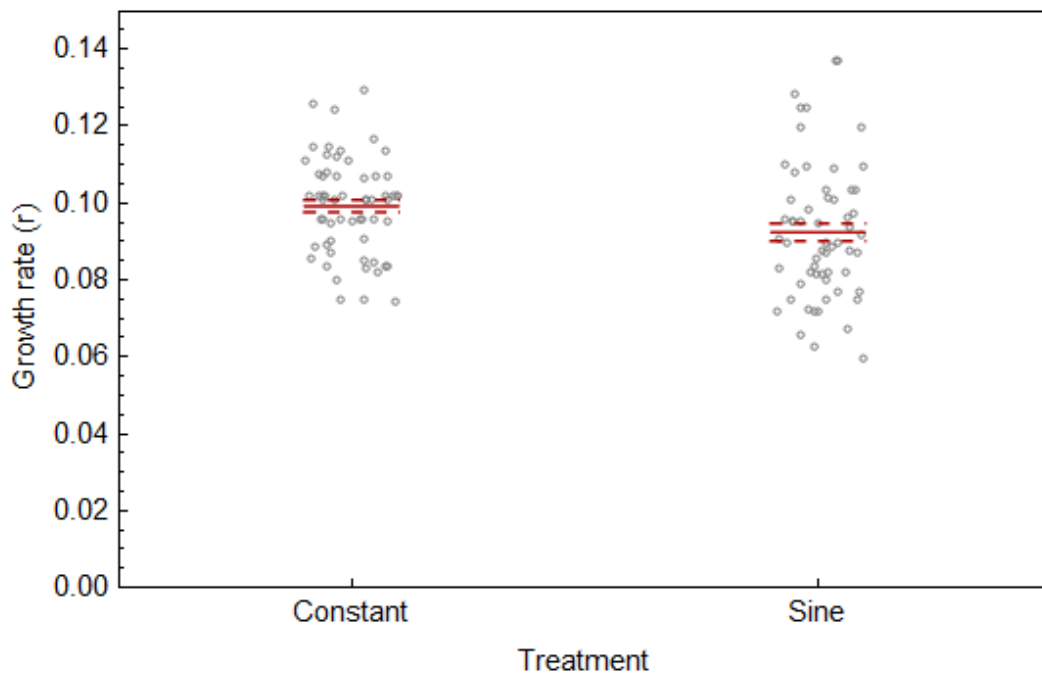


Figure 5. This figure compares the growth rates between constant 13°C and sine wave fluctuations around 13°C.

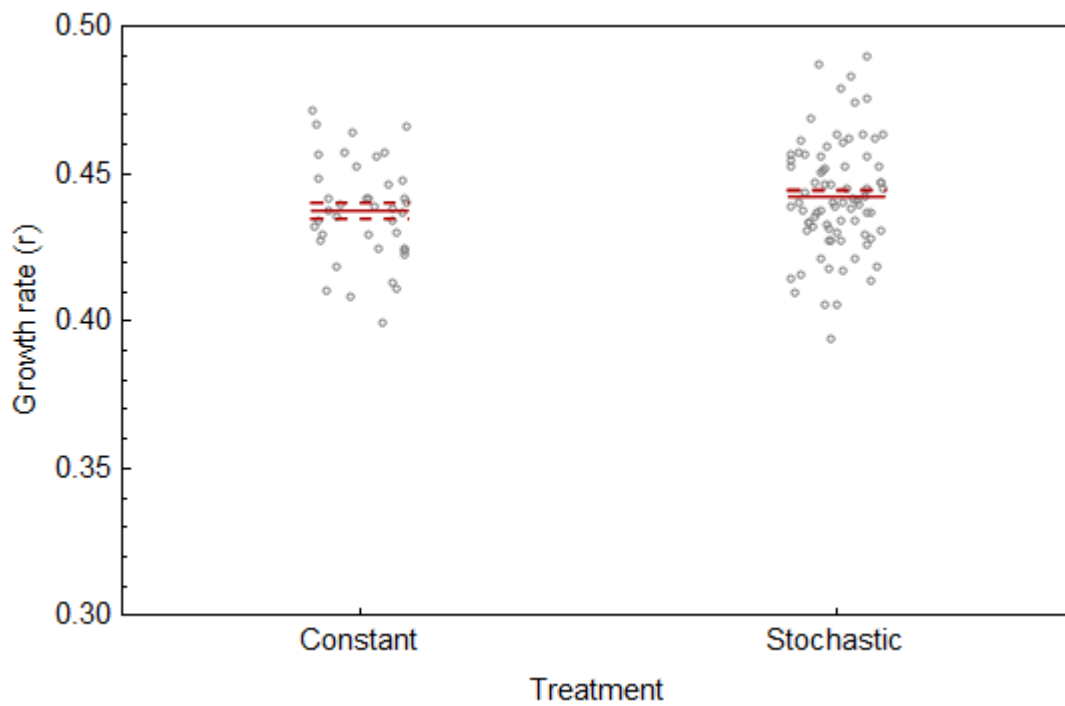


Figure 6. The figure above shows the growth rates at constant 28°C compared to growth rates with stochastic fluctuations around 28°C.

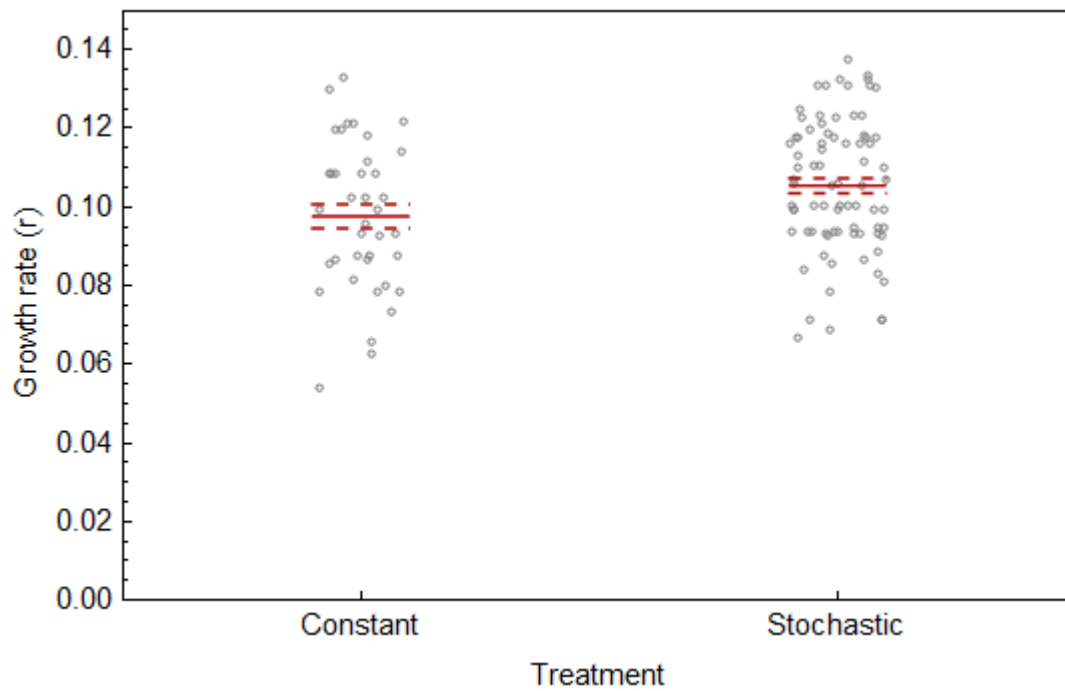


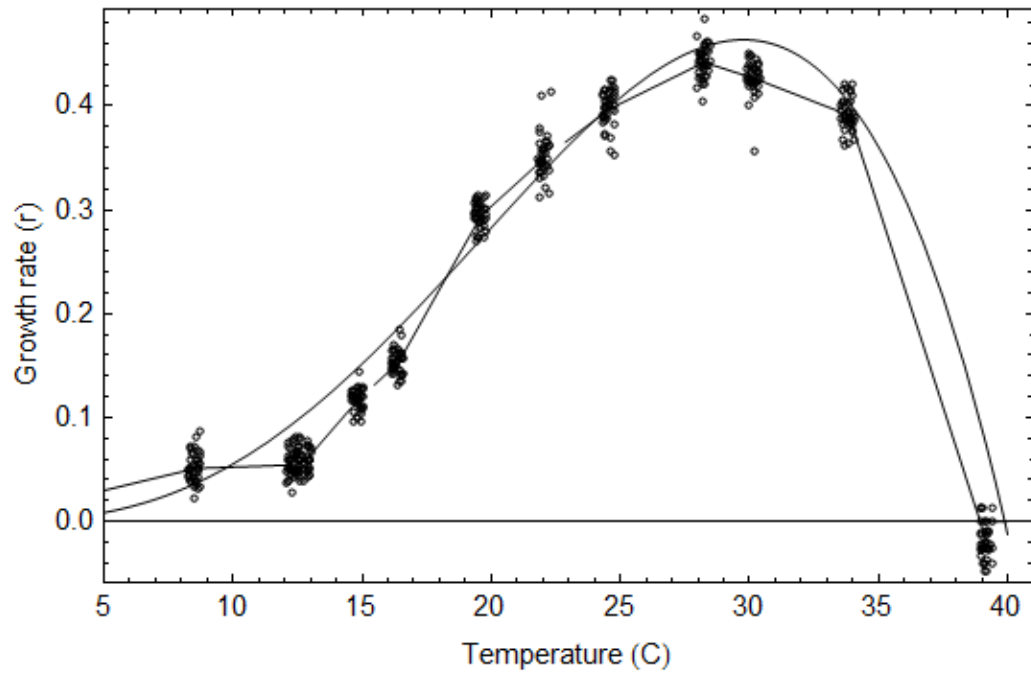
Figure 7. The figure above shows the growth rates at constant 13°C compared to growth rates with stochastic fluctuations around 13°C.

Appendix

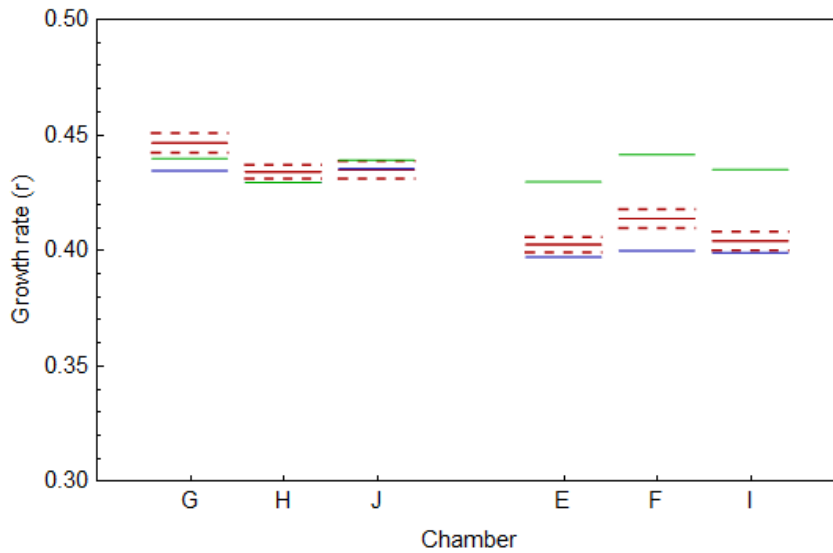
I. Nutrient Water Ingredients

Substance	Concentration in Stock (g/L)
NaNO ₃	8.50
KH ₂ PO ₄	1.34
MgSO ₄ · 7H ₂ O	15
CaCl ₂ · 2H ₂ O	7.2
Na ₂ CO ₃	4.0
H ₃ BO ₃	1.0
MnCl ₂ · 4H ₂ O	0.20
Na ₂ MoO ₄ · 2H ₂ O	0.010
ZnSO ₄ · 7H ₂ O	0.050
CuSO ₄ · 5H ₂ O	0.0050
Co(NO ₃) ₂ · 6H ₂ O	0.010
FeCl ₃ · 6H ₂ O	0.17
Na ₂ -EDTA 2H ₂ O	0.28

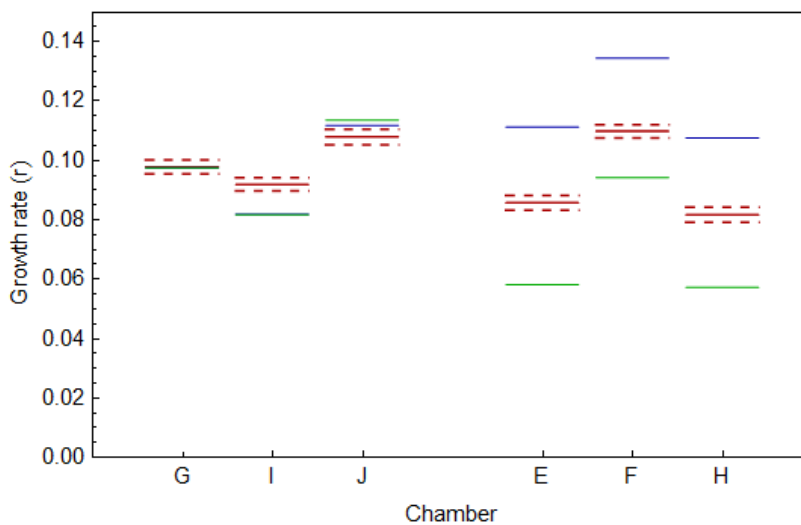
II. Interpolation of growth rates at constant temperatures



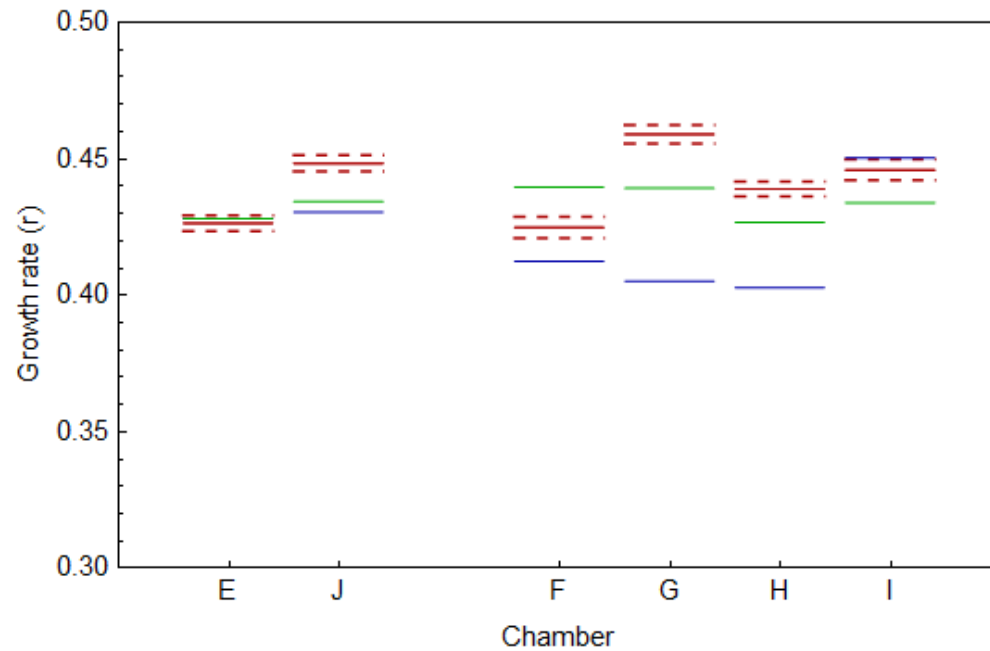
The above figure shows a linear interpolation of the data in contrast to the constant temperature growth curve (Figure 1).



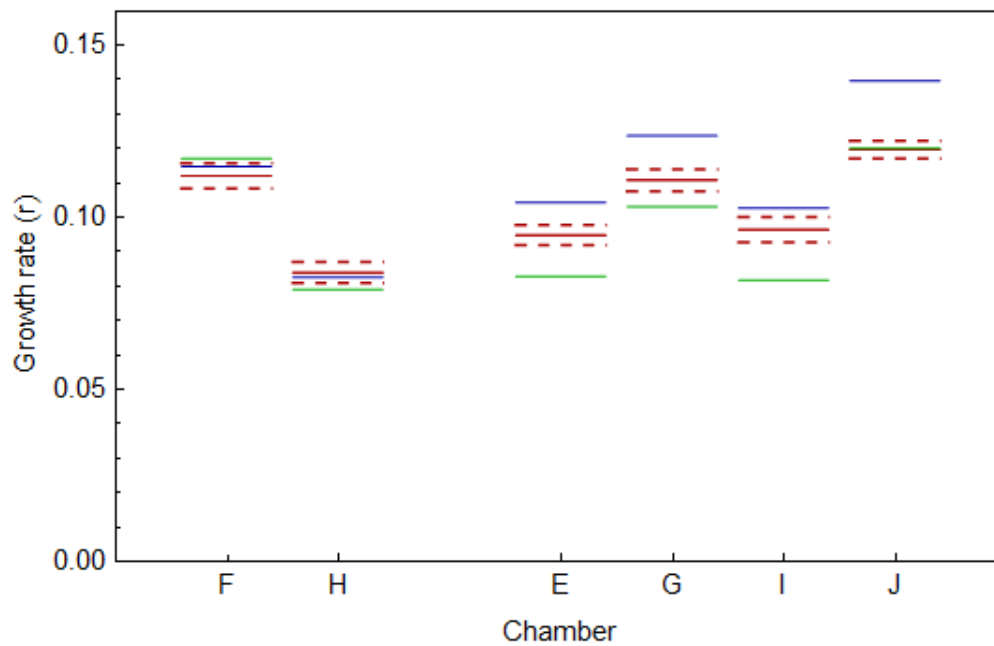
This figure shows the quantitative predictions from the interpolation of the first trial comparing growth rates at a constant 28°C and sine wave temperature fluctuations around 28°C. Each letter represents a chamber, and the solid red lines are the mean growth rates for those chambers. The dotted red lines are the standard error. The 3 chambers to the left are the constant temperature chambers while the chambers to the right are the fluctuating temperatures. The blue lines are the predicted growth rates for each chamber, which was found by first calculating the growth rate at every temperature point recorded by the HOBO loggers (every 5 minutes) and then averaging the growth rates. The green lines are the predicted growth rates for each chamber found by averaging all the temperatures and then calculating the growth rate. The green line corresponds to what would be expected if the duckweed was grown at constant temperatures.



This figure shows the quantitative predictions from the interpolation of the second trial comparing growth rates at a constant 13°C and sine wave temperature fluctuations around 13°C.

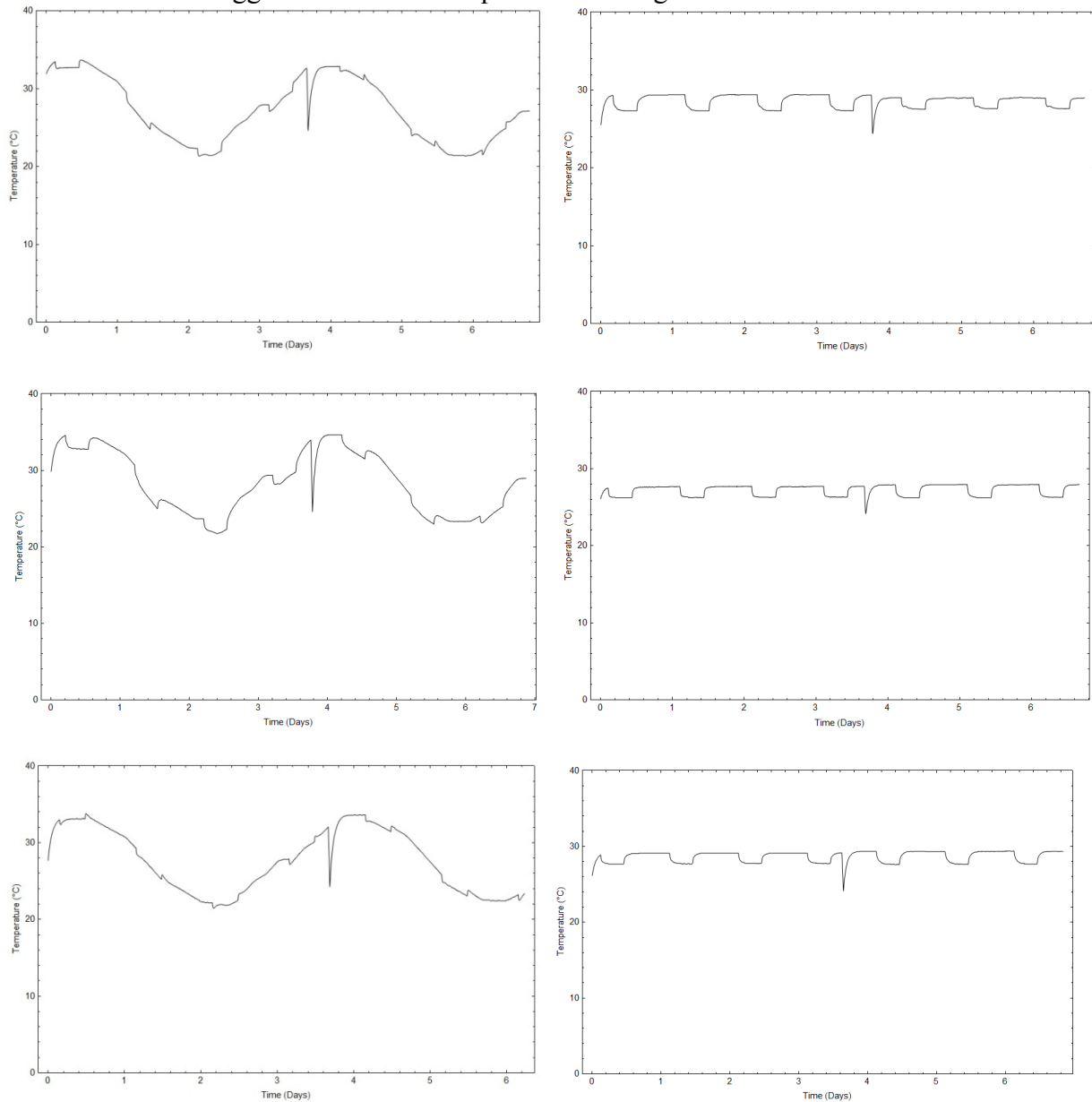


This figure shows the quantitative predictions from the interpolation of the first trial comparing growth rates at a constant 28°C and stochastic temperature fluctuations around 28°C.

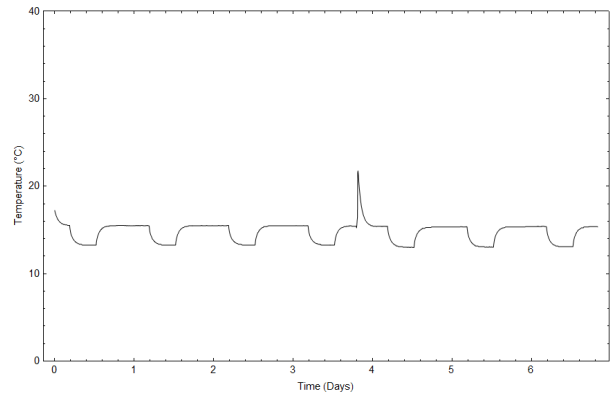
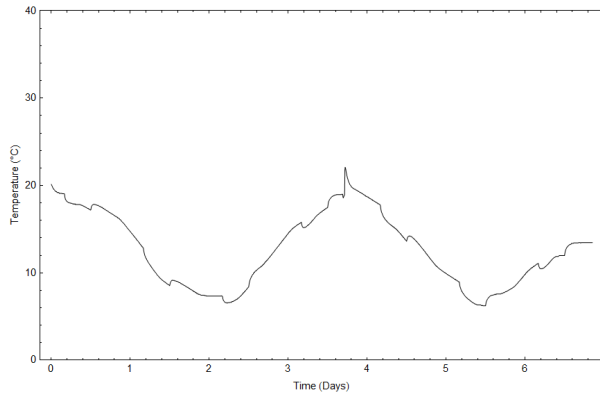
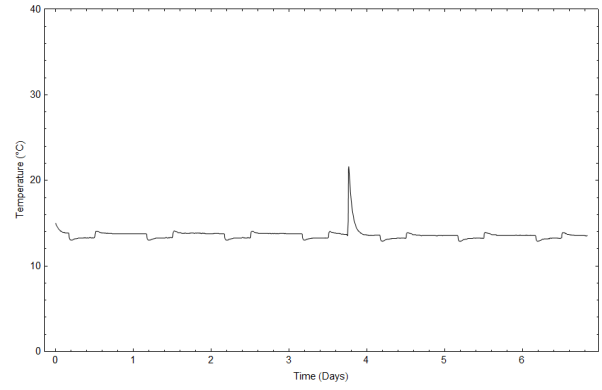
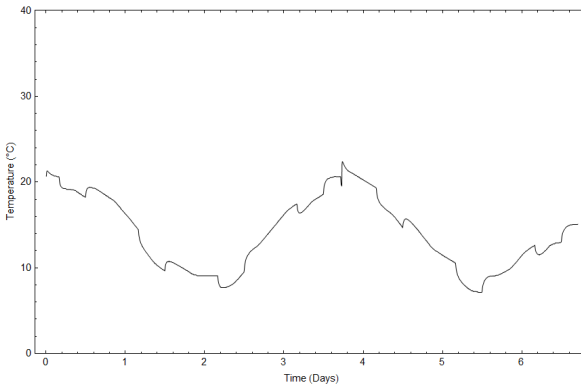
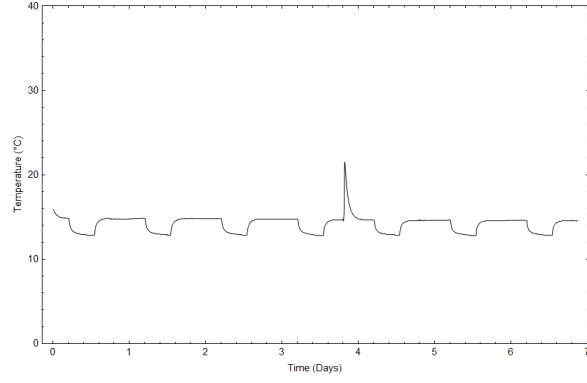
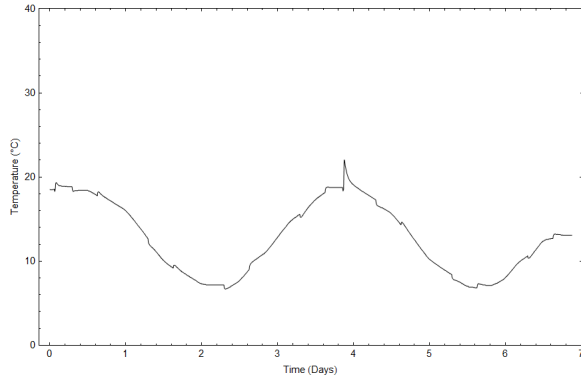


This figure shows the quantitative predictions from the interpolation of the first trial comparing growth rates at a constant 13°C and stochastic temperature fluctuations around 13°C.

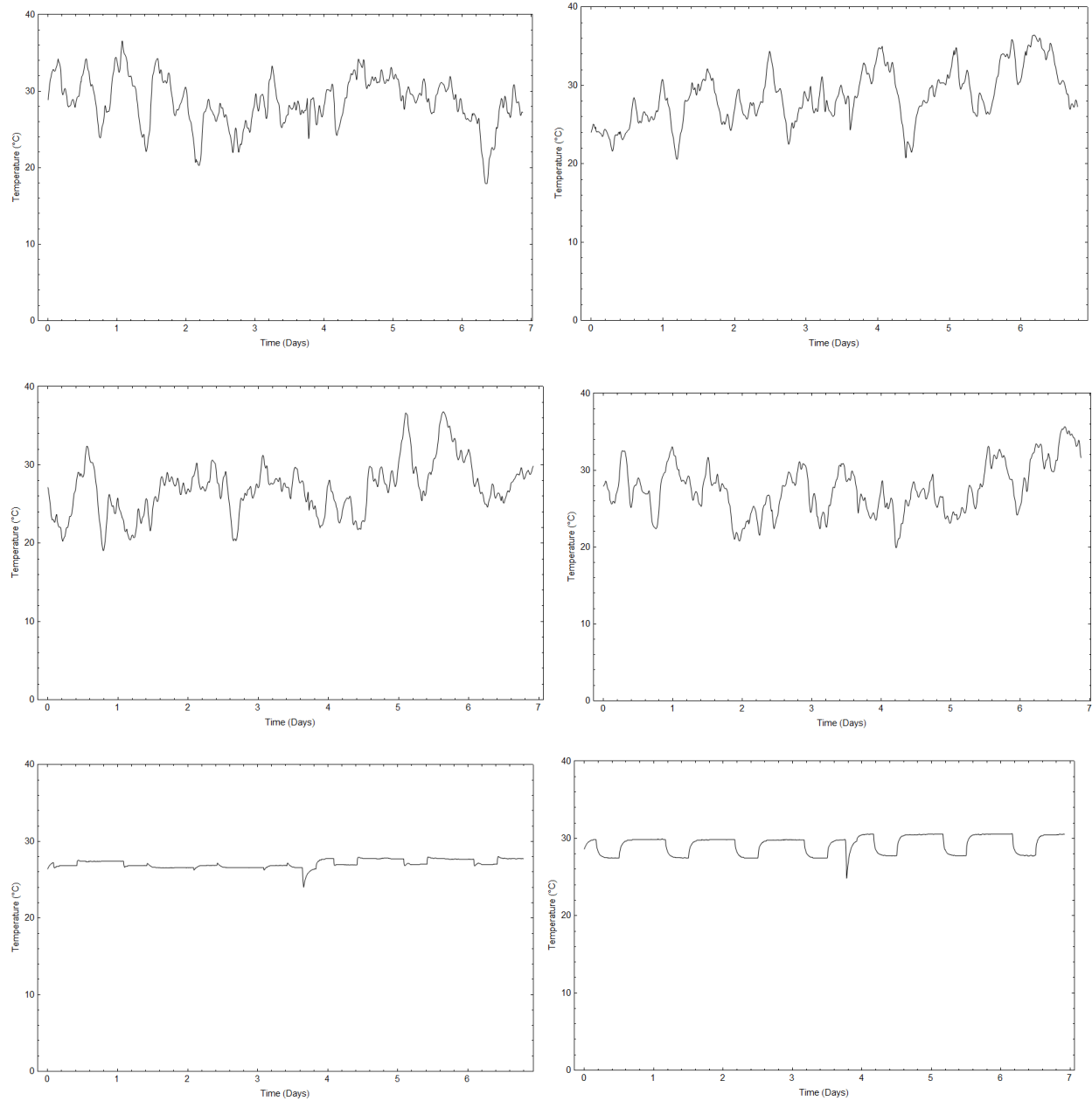
III. Hobo Logger Data-Actual temperatures during the second week



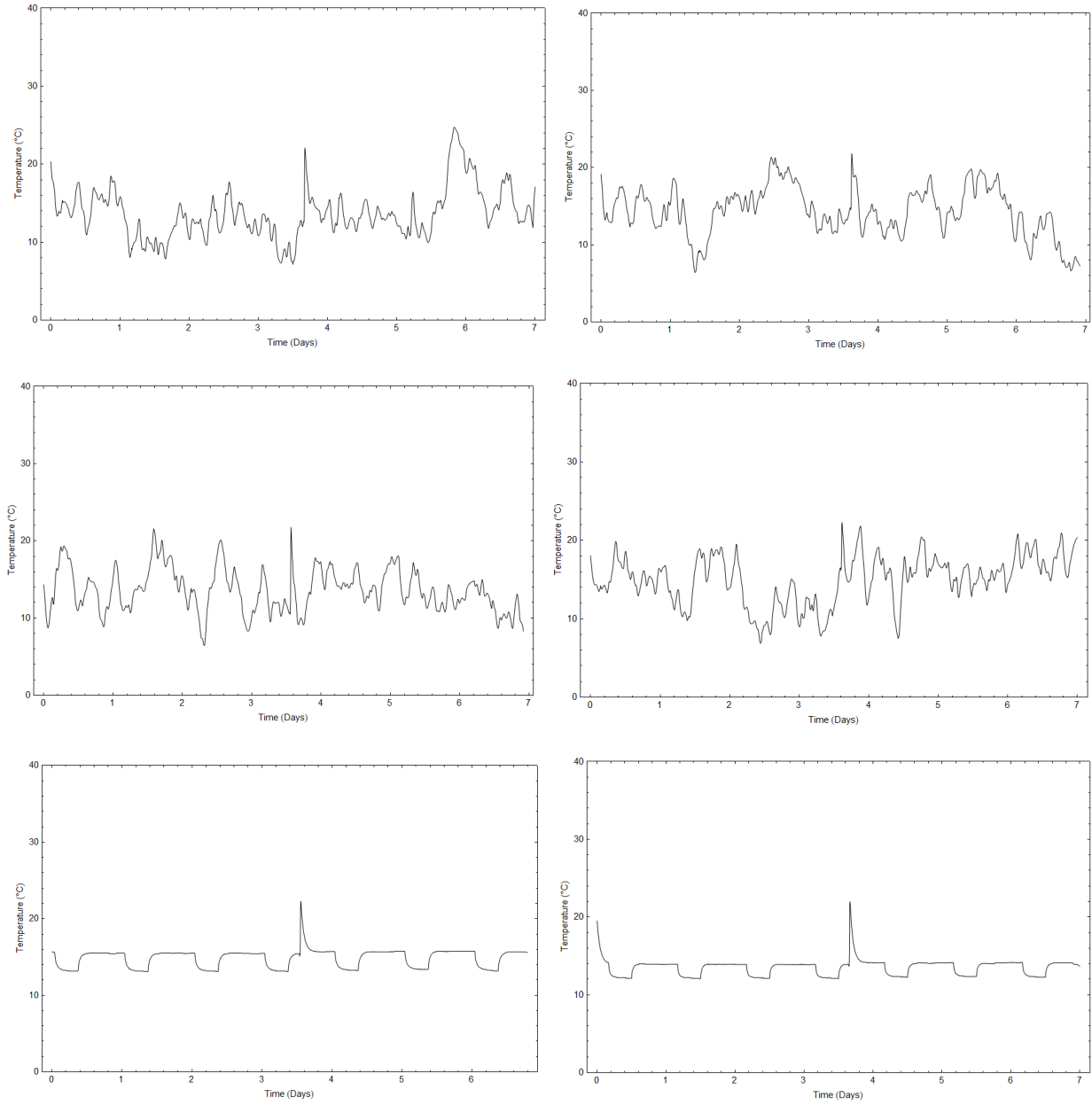
The figures above show the actual temperatures of all 6 chambers during the second week when the per capita growth rate was measured. The figures in the left column show the sine fluctuations around 28°C while the figures in the right column show a constant temperature of 28°C. The noticeable spike in the data around 3.5 days is when nutrient replacement was performed. The other minor fluctuations are caused by when the lights turn on and off and the temperature has to adjust accordingly.



The figures to the left show the actual temperatures in the chambers for the sine wave fluctuations around 13°C. The figures to the right show the actual temperatures in the chambers when they were set at a constant 13°C.



The top 4 figures show the actual stochastic temperature fluctuations of the chambers around 28°C. The chambers with the stochastic temperature fluctuations each had a unique set of data points that were pink-shifted. The bottom 2 figures show the actual temperature of the chambers set at a constant 28°C.



The top 4 figures show the actual stochastic temperature fluctuations around 13°C in the chambers. Each chamber with stochastic temperature fluctuations had a unique set of data points. The bottom 2 figures show the actual temperature of the chambers set to a constant 13°C.